THE EFFECT OF HCG TREATMENT ON DAY OF MATING ON PREGNANCY RATE AND REPRODUCTIVE PERFORMANCE OF SHEEP

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Abstract:

The present study was conducted to determine the effect of human chorionic gonadotrophin (hCG) treatment on on ovarian function, pregnancy rate and reproductive performance in sheep. The estrus was synchronized with two injunctions of PGF2α at 11 days apart. As hCG given on day of mating increased the plasma progesterone concentration. HCG treatment improved the reproductive performance through embryo development embryo growth showed that it had embryotrophic effects, as crow-rump length, amniotic sac width and number of caruncles were increased ($P<0.05$). The ovulation was greater in hCG treatment compared to control. Lambing results indicated that litter size and twins and triplets birth tended to greater following to hCG treatment ($P<0.05$). The improvement in litter size may be either due to increase in ovulation rate or decrease in embryonic mortality. It was found that post-natal mortality has a reasonable contribution to economic losses and its control may result in enhanced production of meat and milk.

Keywords: human chorionic gonadotrophin, ovarian function, pregnancy rate, reproductive performance

Introduction

Sheep are primarily raised as a source of food, wool and skin, as also for immediate cash. The reproductive efficiency of sheep in the county appears to be low, with low rate of twining and no proper breeding practices in place.

To increase the productivity, flock management techniques employ a variety of methods, including estrus synchronization, artificial insemination and *in vitro* fertilization in sheep (Wildeus, 1999; Abecia *et al*., 2001; Dixon *et al*., 2006; Turk *et al*., 2008; Khan *et al*., 2009; Lashari and Tasawar, 2011, 2013).

For more than half a century, attempts have been made to synchronize the period of sexual receptivity, or estrus in farm animals. Estrus synchronization can save labour, and is a key component in AI programmes (Knights *et al*., 2006). The technology of AI reduces the incidence of venereal diseases and greatly increases the genetic merit (Foote, 1999).

The reproductive efficiency of sheep is limited by pre-implantation losses and there is evidence to suggest that the majority of these losses may be preventable, as only a small percentage of embryos are inherently non-viable (Wilmut *et al*., 1986). Embryonic mortalities have been extensively studied on ovine (Khan *et al*., 2009).

In sheep, some 20-40% of ova are not represented by offspring (Quirke *et al*., 1986) and in cattle these losses may also reach upto 40%, the embryo lost in first month of gestation (Diskin and Sreenan, 1980). Fertilization failure accounts for only 5-10% of losses (Wilmut *et al*., 1986), whereas the majority of these are the result of embryonic mortality (Beck *et al*., 1996; Khan *et al*., 2006). As these losses occur after day 12 of pregnancy, they can result in a significant extension of the inter-estrus interval, which could prevent re-mating and lead to barrenness (Beck *et al*., 1996).

Inadequate luteal function is a major cause of pre-implantation losses in sheep (Wilmut *et al*., 1986; Ashworth *et al*., 1989; Nancarrow, 1994; Khan *et al*., 2009). These losses can be prevented by supplementing with progesterone during early pregnancy (Nancarrow, 1994) or by stimulating luteal function with gonadotrophins on day of mating (Khan *et al*., 2003) and day 12 of pregnancy (Beck *et al*., 1994; Khan *et al*., 2007; Khan *et al*., 2009).

As hCG has LH-like activities (Kinser *et al*., 1983; Schmitt *et al*., 1996) can stimulate oocyte maturation and ovulation. It may be possible to obtain a similar improvement in fertility using hCG instead of GnRH agonist. From practical point of view hCG treatment should be less expensive. A supplementary injection of hCG given to ewes at the time of mating or day 12 post-mating may improve fertility (Khan *et al*., 2003; Khan *et al*., 2009). This approach is supported by earlier work in goats (Fonseca *et al*., 2005) and cattle, hCG injections were used to improve fertility (Mee *et al*., 1990; Stevenson *et al*., 1990).

It has been shown that multiple injections of hCG (100-300 IU) on days 4, 11, 12, 13 post-mating increase plasma progesterone concentrations and conception rate in sheep induced to breed during lactational anestrus (Kittok *et al*., 1983). Single injection of hCG (100 IU) before the time of maternal recognition of pregnancy may improve conception rate in sheep mated at spontaneous estrus (Nephew *et al*., 1994). The pregnancy rate was inversely correlated with the duration of the growth of the ovulatory follicles (Vinoles *et al*., 2001; Dixon *et al*., 2006). To stimulate follicle development and number and afterwards to increase the ovulation rate and litter size, gonadotrophins such as PMSG (Gordon,1997; Dogon and Nur, 2006), hCG (Khan *et al*., 2009), eCG, FSH (Boscos *et al*., 2002), and GnRH (Cam and Kuran, 2004; Khan *et al*., 2007; Lashari and Tasawar, 2010) co-treated with intravaginal sponges. It has been reported that the use of the GnRH- $PGF_{2\alpha}$ combination in ES (Ataman and Akoz, 2006; Lashari and Tasawar, 2010), and progestagen together with post-mating GnRH administration (Cam and Kuran, 2004; Khan *et al*., 2006) positively effect the fertility parameter s of sheep.

The objective of this study was to investigate the effect of hCG given on day of mating has any effect on luteal function, embryo viability, placentation and reproductive performance of sheep.

Materials and Methods

This experiment was designed to determine the effects of hCG treatment on the day of mating (day of estrus = day 0) by looking at its effect on luteal response and embryo survival in animals mated at a synchronized estrus. Thirty two Lohi sheep 3- 4 years old were divided into two groups balanced by weight using a stratified system of randomization (saline, n= 16; weight 44±0.25kg, hCG, n=16; weight 44.5 ± 0.12kg). After estrus synchronization the hCG treatment group was treated with 300 IU (Pregnyl, N. V. Organon Oss, Holand) intramuscularly. All the animals were put to raddled rams (1 ram per 10 animals) of proven fertility and 48 h after the introduction of the rams the identification numbers of rump marked (mated) animals was recorded. Any ewe not sufficiently rump marked was excluded from the experiment and subsequently, Blood samples were collected from these animals by vein puncture at 10.00 a.m. from day 2 to 16 (both days inclusive).

On day 25 post-mating the animals were slaughtered and their reproductive tracts were recovered for examination. Conceptuses were carefully removed from gravid uteri post-slaughter. Embryo weight (g) crown-rump length (mm) and amniotic sac width/length were determined. The viability of each embryo was assessed from its weight and crown-rump length. The ovulation rate for each group was determined from the number of CL present. The weight of each CL was also recorded. Moreover, the number of caruncles forming placentomes in each uterine horn was also counted. Only animals with viable embryos were considered pregnant and animals containing degenerative embryos were categorized as non-pregnant, excluded from the analysis.

Plasma concentrations of progesterone and estradiol were assayed in all the blood samples collected from days 2-16. The limit of the sensitivity was 0.3 ng/ml and inter and intra-assay coefficients of variation were 12.6 and 7.1%, respectively. Following determination of the progesterone concentrations was calculated for each animal within each group. From this, the daily mean plasma concentrations of progesterone between days 2-16 was determined.

Statistical analysis

The results were expressed as percentages and Mean ± SEM. Comparisons of different types of percentages were made by Chi-square (χ^2) test. The data for plasma progesterone and oestradiol concentrations were analysed using paired t-test. $P<0.05$ was taken as statistical significance.

Results

The results of conceptus growth and development are given in Table 1. Out of 32 ewes put to the ram (Control= 16; hCG day 0 =16), were properly mated as indicated by rump marks. At slaughter there was no difference in pregnancy rates and ovulation rate was higher in hCG day 0 treated groups as compared with control. Of animals carrying viable embryos, most were pregnant with singltons and there was significant difference in number of embryos recovered (control = 17; hCG day0= 21) between treatment groups. Moreover three ewes in the control group and four animals from (hCG do) were pregnant with twins. Furthermore two animals from hCG day0 were pregnant with triplet. The litter size was significantly higher in hCG treated as compared to control.

In the ewe pregnant with viable embryos, there was tendency ($P<0.05$) for amniotic sac length and embryo weight to be greater in hCG group. Also, the crown-rump length and amniotic sac width were greater ($P<0.05$) in the hCG treated group when compared with controls. Furthermore, there was a significant ($P<0.05$) increase in the number of caruncles forming placentomes in both uterine horns in the hCG groups compared to the control group. It was evident from examination of the reproductive tracts post-slaughter that some early embryo loss had occurred in the ewe belonging to both treatment groups, classified as non-pregnant. This was concluded from the presence of degenerative embryos or by the presence of dark eroded caruncles, which are indicative of pregnancy failure at an early stage.

Plasma progesterone

Plasma progesterone concentrations in saline or hCG treated on day of mating shows there was no significant difference ($P>0.05$) between saline or hCG treated day of mating. Progesterone levels in day mating animals were observed to increase faster rate than in saline treated animals, however days after 15, levels began to stabilize at between 3 and 5 ng/ml in saline or hCG day of mating. Finally, on day 15 plasma progesterone concentrations for hCG group were higher (control=3.8; hCG day 0= 4.9ng/ml) than in control group.

Plasma oestradiol

The results of plasma oestradiol concentrations of ewes in control or hCG treatment groups was similar in saline or hCG treated on day of mating between days 9 and 12. Plasma oestradiol concentrations on day mating animal were observed to increase faster rate than saline. The response to hCG on day mating treatment was significant ($P<0.05$).

Table 1. Reproductive performance of sheep given saline or hCG at synchronised estrus and slaughtered at day 25 post-mating.

		Control	HCG
No. mated at treated estrus		16	16
No. (%) of animal pregnant at slaughter		14 (87)	13 (81)
No. of CL in pregnant animals		19	25
Rate of ovulation		1.36[a]	1.92[b]
No. of embryos recovered:	Single	11	07
	Twin	03	04
	Triplet	00	02
	Total	17	21
Litter size		1.21[a]	1.62[b]
Embryo mortality (%)		10.5[a]	16[a]
Mean amniotic sac length (mm ± SEM)		14.7 ± 0.4	15.1 ± 0.5
Mean crown-rump length (mm ± SEM)		11.93 ± 0.23[a]	12.59 ± 0.21[b]
Mean amniotic sac width (mm ± SEM)		11.41 ± 0.36[a]	11.98 ± 0.28[b]
Mean weight of viable embryo (g ± SEM)			
	Single	0.178 ± 0.003	0.182 ± 0.005
	Twin	0.151 ± 0.007	0.169 ± 0.009
	Triplets	0.138 ± 0.006	0.157± 0.007
Mean weight of CL (g ± SEM)		1.41 ± 0.15	1.5 ± 0.12
Mean no. of caruncles (± SEM)	Left horn	46.5 ± 3.9[a]	52.6 ± 4.1[a]
	Right horn	47.3 ± 3.5[a]	53.2 ± 4.3[a]
	Total	93.8 ± 6.8[a]	105.8 ± 7.1[a]

Values with different superscripts within same row are significantly ($P < 0.05$) different.

DISCUSSION

The hCG has been previously used to improve the reproductive efficiency in cows given single injunction (Peters *et al*., 1992; Rajamahendran and Sianangama, 1992; Sianangama and Rajamahendran, 1992) and in pigs (Bolama *et al*., 1991), and in sheep (Khan *et al*., 2003, 2009). The present study not only reports the use of hCG supplementation prior to insemination increase conceptus growth that enhance fertility in ewes. This would have helped improve embryo survival as larger conceptuses produce more IFN-tau, thereby more effectively suppressing the luteolytic mechanism and allowing more time for the establishment of pregnancy (Nephew *et al*., 1994; Spencer *et al*., 1996). Placentation was also improved in hCG day of mating treated animals, as the number of placentomes was increased. It could be hypothesised that a great number of placentomes resulting in a larger overall surface are might improve attachment of the embryos and therefore reduce embryo losses by improving placentation.

The reproductive performance of the ewes was lower after synchronised estrus than would be expected for ewes mated naturally (Gordon, 1997). This may have been because the animal used in this study originated from a commercial flock and were not used to handling. Furthermore, their body condition score was not in the ideal, which may have been because they were more than five year old and had not regained sufficient condition after weaning. Human chorionic gonadotrophin treatment appeared to improve embryo survival in hCG treated day of mating animals having more triplet birth than the saline treated control.

The pregnancy rate was similar in animals slaughtered at day 25; however there was larger litter size in the hCG treated group which is consistent with a previous report in the ewes given hCG treatment (Zamiri and Hosseini, 1998; Khan *et al*., 2003, 2009). Furthermore, it is also evident from the results that hCG treated animal produces a higher proportion of twins and triplets than the animals in control group. The latter was probably the results of a significant ($P<0.05$) increase observed in hCG treated animals, slaughtered on day 25. Such an increase in ovulation rate might have resulted from a faster rate of maturation or ovulation of immature follicles (Kinser *et al*., 1983; Khan *et al*., 2009) induced by prolonged effect of hCG due to its longer half life than the natural LH (Khan *et al*., 2003).

The improved conceptus growth might be expected to increase birth weight. However, there was tendency for single, twin and triplet birth to be heavier in both hCG group than control. The significant ($P<0.05$) difference was observed in placentome numbers in day 12 hCG treated animals. This effects the conceptus growth and may therefore, have increased the chances of survival. And indeed, the litter size was significantly higher in the hCG treated animal compared to the controls. Furthermore a study by Khan *et al*. (2003) demonstrated that ovarian steriodogenic response to hCG treatment on day 12 post-estrus is significantly higher compared to control. Similar findings were reported by Khan *et al*. (2003). Fonseca *et al*. (2005) reported that hCG treatments have no effect on reproductive performance of goats. However it possible that the hCG supplementation may be more effective in enhancing the reproductive performance or reducing embryonic mortality in these climatic conditions.

The results of plasma progesterone concentrations were similar to that reported for ewes (Ashworth *et al*., 1989) does (Fonseca *et al*., 2005). Plasma progesterone concentrations were between 3-6 ng/ml during the luteal phase, indicating that luteal function in the majority of animals was higher in both hCG treated as compared to control. Furthermore, considering the significant difference in ovulation rate (control=1.36; hCG day0=1.92; hCG day12=1.84) between the three groups, the plasma concentration of progesterone were different between treatment groups as it has been shown that progesterone concentrations differ between single and twin ovulators (Khan *et al*., 2009).

Finally, as hCG has both FSH and LH activity (Kinser *et al*., 1983; Khan *et al*., 2003), this combination could have forced the ovulation of immature follicles, which normally would have become atretic. The resulting CL could be less steriodogenic as it has gone through a lesser degree of lutinization and consequently secretes less progesterone (Ashworth *et al*., 1989). Although local concentrations of progesterone were not measured in the present study, there is evidence to suggest that the local levels were higher in pregnant hCG treated ewe as indicated by the increase in conceptus length/width and weight in the hCG treated group (Khan *et al*., 2003).

Conclusion

In conclusion the results of present study indicate that hCG treatment given at synchronized estrus increased the plasma progesterone concentration, ovulation rate. This treatment may improve embryo survival thus suggesting that hCG treatment given on day of mating has a potential to improve reproductive performance.

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